ON THE NUCLEOLI IN THE SOMATIC AND GERM CELLS OF PEDICELLINA AMERICANA.

LOUIS I. DUBLIN.

In the course of a work on the germ cells of *Pedicellina americana*, just published, (Dublin, '05), my attention was called among other matters to the study of the character of the nucleolus, not only in the various stages in the differentiation of the germ cells, but in the somatic cells as well. Thus, in the resting condition of the cell, the nucleoli are nearly everywhere in pairs and are situated at opposite points near the nuclear membrane. This arrangement is so constant, as are also the later processes through which these bodies pass, that the possibility was immediately suggested that *Pedicellina* might be one more instance among the many cited by Häcker, '02, where there is a distinct outward expression of the internal independence of the paternal and maternal elements, not only in the nuclei of the early somatic cells, as is most clearly observed in several forms, but also in the early germ-cells. It was therefore determined to put this whole question to a test by an examination of the cells throughout the whole life cycle, *viz.*, to observe whether the condition of the chromatin was actually in accordance with the conditions so strongly suggested by the appearance and behavior of the nucleoli.

For this purpose *Pedicellina* presents very favorable conditions. In the first place, owing to the comparatively small size of the polyps, the various tissues including the ovaries and testes, can be brought into the same microscopic view, thus affording very close comparison of these structures throughout. In the second place, and more important, owing to the nature of the budding process and the internal development of the embryo, the cells of all the stages of the life history are easily accessible for study with the exception of a short and, for our purpose, rather unimportant period during metamorphosis when the free swimming larva leaves the brood-pouch and becomes attached. Such a study of the entire life history is, finally, all the more important

in the light of the discussion on the nature of the nucleoli and of the relation which the latter bear to the chromatic substance. I shall then, first, review the character of the nucleoli throughout the life history and, second, limit myself to the nucleolus of the growing oöcyte.

I.

The youngest polypides at my disposal were those which had but lately passed through their metamorphosis (Fig. 1). These in many cases do not as yet show any traces of the budding stolon and have certainly no genetic connection with the colonies among which they are found. There is still no apparent distinction of sex and the internal structures are at the beginning of their development. Of the digestive system, only the middle portion has definitely formed. The atrium or vestibule is represented by a small space which has as yet no connection with the outside. Surrounding the gut and vestibule and filling the space between these parts and the body wall are masses of embryonic cells which later give rise to the ovary or testis to the nervous and excretory systems, the œsophagus and rectum, the tentacles and the few muscle bands. These embryonic cells are all of one main type and cannot as yet be distinguished very positively from the primitive germ-cells which lie in their midst. One character, however, stands out strikingly. The comparatively large nuclei, in the great majority of cases, contain each two nucleoli. These two bodies are located symmetrically with respect to the halves of the nucleus. In the remaining cases there is but one nucleolus and this one is almost invariably of larger size than either of the two and is located at or very near the center of the nucleus.

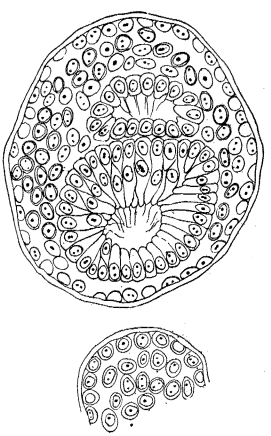

FIG. 1.

In a somewhat later stage, the stalk is longer and the internal organs begin to show something of their permanent form. The primitive germ-cells, which can now be distinguished very clearly, have taken their final place between the "liver-cells" and the

floor of the atrium and there multiply very rapidly. This increase is much more pronounced in the male than in the female and gives the first and principal basis for the distinction now present between the sexes. The bi-partite testis, with its spermatogonia, has grown considerably and the latter are mostly in the resting condition. The nuclei are large, being surrounded by a very thin layer of cytoplasm. Within the nucleus, the chromatin, in the form of a reticulum, stains very lightly; indeed, in well extracted iron-hæmatoxylin preparations shows scarcely at all. But what is more important is the almost invariable presence of the one or the two nucleoli. These stain uniformly and intensely black with hæmotoxylin and retain their color long after that of the other cell elements has been extracted. When in pairs, they are placed at opposite points of the nucleus; when single, the nucleolus is found more nearly in the middle. The cells of the other tissues have preserved the same condition of the nuclei as was above described for the younger polyp.

In older testes, many more cells are present (Fig. 2), and these are proved, by the unreduced number of the chromosomes, to be also spermatogonia. Among these are cells of many sizes both

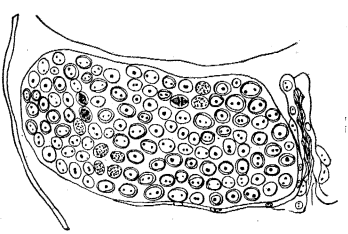

FIG. 2.

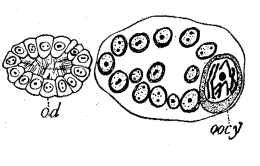

FIG. 3.

at rest and in mitosis from which it is certain that there are several generations of spermatogonia. In tracing the development of the spermatogonium from the telophase of the preceding division, attention is attracted by the appearance of the two nucleoli. At the beginning of the reconstruction there is as yet no trace of them. This stage is soon followed by one where the nuclear membrane has reappeared, the chromatin being almost entirely unaffected by hæmatoxylin. At two opposite points of the nucleus there now appear the two nucleoli as yet very small but

deeply staining, very much like the granule of a centrosome. It is important to observe, that the number is at this point always two, the one larger nucleolus occurring only in the later stages of spermatogonial development. The chromatin soon increases in staining power, the nuclear membrane stands out more clearly and the two nucleoli increase in size until they become very conspicious. In the Auerbach preparations, the green chromatin reticulum near the nuclear membrane stands out rather clearly from the two red plastin nucleoli. These, at this point, approach the center of the nucleus and finally fuse into one larger nucleolus. All the stages in the approach of the two are to be found in one microscopic field in almost any testis. The appearance of these bodies as they come into closer relation, indicates that there is a flowing of their liquid substance toward a common center. In this way is produced a fine strand, deeply staining, which connects them before they have fused. We can now more readily understand the prevalence of the single nucleoli in resting spermatogonia. They represent the two, which, originally distinct, have now fused. It must here be added, however, that there is some doubt whether the two always fuse into one. There is evidence for the possibility that, in some few cases at least, the two remain distinct until the formation of the next spindle figure when they disintegrate.

The chromatin by this time stains much more intensely and takes the form of definite chromosomes. These may now move from the periphery and come into closer relation with the one or the two nucleoli. This connection is, however, always a secondary one, and in no case represents the origin of chromosomes from chromatin nucleoli. There can be little doubt of this conclusion, for on staining with Auerbach's fluid the chromatin stains intensely green while the nucleoli are always red. The same decisive results were obtained with the use of the Borel stain.

The oögonia at first resemble the spermatogonia very closely (Fig. 3) but can be distinguished, even at an early period, by their smaller number. The chromatin goes through exactly the same transformation in the several generations as do also the two nucleoli. In their early appearance at or near opposite points on the newly formed nuclear membrane, in their subse-

quent growth and movement toward the center, and finally, in their fusion, the resemblance is quite complete. We may therefore infer that whatever be the significance of the regularity of the occurrence of these bodies in the males must also hold in the case of the females.

This striking condition of the nucleoli of the primary germ-cells (Ur-genital Zellen) and of the several generations of spermatogonia and oögonia can be no mere chance occurrence. In the testes of older individuals, where some of the spermatocytes have already arisen, nearly every resting spermatogonial cell shows the primarily double or the secondarily fused condition of the nucleoli. This period closes, however, with the spermatogonic cycle since the spermatocytic divisions follow without an intervening resting stage. In the ovary, where the last oögonial division is followed by a long period of growth, the young oöcytes also show the same condition of the nucleoli that has been described for the oögonia. These either persist separately or fuse, but, unlike the nucleoli of the earlier germ-cells, become the composite structures generally found in the nearly matured egg. To this, however, we shall turn below.

As was pointed out in the discussion of the young polypides, where the internal tissues are not as yet completely differentiated,

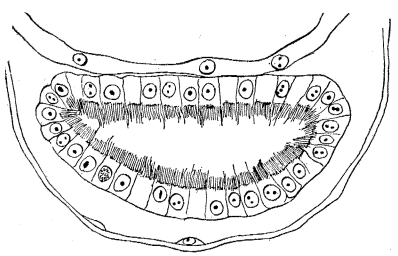

FIG. 4.

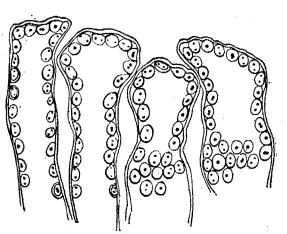

FIG. 5.

the primary germ-cells are not to be distinguished either in size or form from the other embryonic cells. These latter also show the same double nucleoli and act in other respects as do the germ-cells. From this we might expect to find similar conditions in the several tissues of the body to which the embryonic cells give rise, and such is, in fact, the case. In polyps, old or young, in males or in females, every organ shows the same con-

stitution of the nuclei. In the highly-modified "liver-cells," in the epithelium of the gut (Fig. 4), in the cells of the atrium and tentacles (Fig. 5), the primitive conditions are alike preserved.

From the universality of the occurrence of this phenomenon, it is difficult to escape the conviction that we are here concerned with conditions of considerable importance in the ultimate constitution of the nucleus. We must now inquire what light the fertilization and the early cleavage stages will throw on the problem; for it is here that the constitution of the nucleus ought most readily to be made out. In the first place, the two pronuclei do not completely fuse in the act of fertilization. In this regard, *Pedicellina* recalls the condition found by many observers in other forms. Indeed, the individual chromosomes are often formed in both pronuclei before their apposition has occurred and in no case is there much, if any possibility of their fusion during

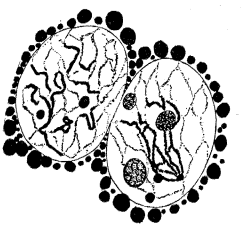

FIG. 6.

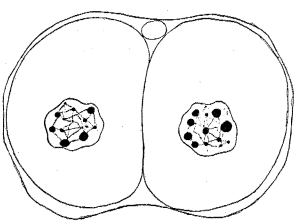

FIG. 7.

fertilization. The nuclei are extremely large and apparently correlated with this is a fact that each contains many nucleoli; the number varying from two to as many as seven or more (Fig. 6). In the 2- (Fig. 7), 4-, and 8-cell stages, the same conditions with reference to the nucleoli exist. As the cleavages go on a little further the conditions become more in accordance with what was observed in the latter somatic stages. The nuclei become successively reduced in number and it is no uncommon occurrence to find in a 16-cell stage as many as half the cells with two nucleoli (Fig. 8). These two may fuse into one, as is the case in the oögonia and spermatogonia already described. In the 32–64-cell stage, the mono- or bi-nucleolate condition is already the all-prevailing one, all the stages in the approximation and fusion of the two into one being present (Fig. 9). From this point forward, I have never found, in any of the many em-

bryos examined, more than two nucleoli and these go through all the typical transformations already described. The same conditions are obtained in all the resting cells of the later embryos up to and including the free-swimming larva (Fig. 10). After

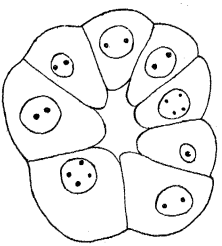

FIG. 8.

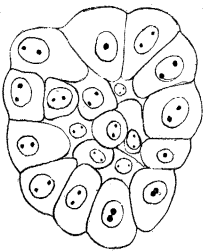

FIG. 9.

the fixation of the latter, and the ensuing metamorphosis, the young polypide, before stolon formation, shows the same interesting conditions. We have thus traced the constitution of the nuclei from the early polyp through the maturer ones, into the formation of the germ-cells and finally, in the new generation, have passed through the cleavage stages up to the formation of the larva and have completed the cycle with the fixed polyp once more.

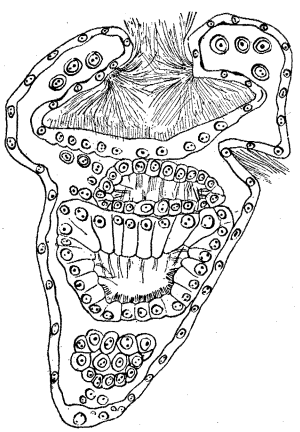

FIG. 10.

From this evidence, coupled with that of Rückert, '95, Häcker, '95–'02, Zoja, '95, Herla, '93, Conklin, '01–'02, and most recently of Moenkhaus, '04, one might at first thought conclude that in *Pedicellina* as in the Copepods, etc., the nucleoli actually express the internal relations of the parental chromatins of the nucleus and in their duality represent the persistent segregation and autonomy of the paternal and maternal chromosomes. This conclusion, however attractive, has on close examination of the evidence not only in *Pedicellina*, but in the other forms as well, a source of serious difficulty. From the recent work of Moenkhaus, '04, above mentioned, it is clear that while the two nucleoli may be very

generally present in the late cleavage stages of the hybrid teleost embryo, the parental chromosomes are positively not separated into two groups. Thus during and after the third cleavage of the egg, the long and the short chromosomes of the two parents of the hybrid mingle together, the spindles never again showing any division into right and left sides with the corresponding separation of the chromosomes, according to the work of Häcker, so marked in the early divisions of the copepod egg. The double nucleolate condition, cannot, therefore, be taken as an indication of the autonomy of the paternal and maternal contributions.

In *Pedicellina*, moreover, the fertilization and early cleavage stages, where the autonomy of the parental chromatins would be most marked if at all present, is not at all in evidence. In spite of the bi-nucleolate condition of the later life history, it is clear from the early stages that the chromosomes of the egg and the sperm have, as in the Fundulus-Menidia hybrid of Moenkhaus, entirely mingled, perhaps in the first cleavage spindle. Thus, also in *Ascaris*, according to Zoja, '95, where the chromosomes of the egg and sperm have mingled as early as the 12-cell stage. In the Copepods themselves, it is not clear that the physiological distinction in the parental sides of the spindles persists after the early cleavages, and thus, the only important evidence for persistent gonomery is, in these types, the constant appearance of the two nucleoli, and this, it is clear, involves an assumption which, in view of the latest work, is full of difficulties. Of still less weight is the occurrence of bilobed nuclei in the late stages, these probably representing little more than an intermediate stage in the fusion of the several vesicles into one resting nucleus. The appearance of the double spirem in the early germ-cells while of greater value is, from the rarity of its occurrence and from the want of proof that the halves are parentally different, also indecisive.

It is therefore much more probable that the parental chromosomes actually mingle among themselves at an early period of development, and that these so-called outward expressions of internal independence are either accidental and unconnected with the chromatin phenomena or are expressive of other conditions

within the nucleus. By this it is not meant that the chromosomes lose their individuality or fuse into one indiscriminate mass. On the contrary, in most forms examined on this matter and in *Pedicellina* particularly, the persistence of this individuality is most marked although it is impossible to distinguish between the paternal and maternal chromosomes now mingled among themselves.

This conclusion is not only more in accordance with the facts but can alone directly explain the possibility of a parental synapsis, *i. e.*, the union of the homologous paternal and maternal chromosomes into pairs, thus reducing the number to one half at the close of the oögonic and spermatogonic cycles in the early germ-cells (Montgomery and Sutton). Were the homologous chromosomes segregated at this period also, this union would be impossible; mingled together, however, as Moenkhaus has actually shown in the teleost and as is very probable in other forms, the union would be readily effected.

What then is the significance of the two nucleoli so constant in the life history of *Pedicellina?* In the present state of our knowledge, when such uncertainty as to the nature and function of the nucleolus still persists, no definite answer can be given. In *Pedicellina* it is clear that they are not chromatin bodies and come only incidentally into connection with the chromosomes. They are plastin bodies in some way, very probably, connected with the waste products of division, as Häcker's theory maintains. Yet, whatever may be the significance of the nucleoli as organs of the cell, they do in some way reflect or represent the activities of the nuclear areas in which they arise. When, therefore, we find in the cells of the cleavage and later stages two nucleoli removed to opposite portions of the nucleus we may perhaps be permitted to infer that these are the outward expressions of the activities of these two portions which together compose the nucleus, and if an interpretation of parental nuclear autonomy be at all justified by the facts, and this is very questionable, I should suggest that it is much more probable that this autonomy applies to the paternal and maternal sap or ground substance in which the chromosomes are distributed.

In this connection, there is one more consideration to which attention must be drawn. In the spermatid of *Pedicellina*, the

chromatin appears in two masses; one localized at the anterior, the other at the posterior end of the now elongated sperm head, there being a non-staining middle space between (Fig. 11). On such a condition as this one might extend to this post-maturation stage the same interpretation that was criticized above. Indeed Häcker would so conclude. In his '02 work, referred to above, this author is quite ready to interpret the presence of two nucleoli in the matured sperm head as an indication of the persistent autonomy of the parental chromatin halves, even until this period. The only uncertainty, as he admits, in the Copepods under consideration (*Diaptomus* and *Heterocope*) being the presence of three or four nucleoli; nor are these placed at two opposite points of the nucleus as his hypothesis of gonomery demands.

FIG. 11.

In the matured egg, however, he finds absolutely no difficulty in accepting this conclusion. Speaking of the chromosomes at the beginning of the second maturation division, he states, p. 343: "Die 12 neuformirten bivalenten Elemente . . . werden durch den Reduktionsakt auf der zweiten Richtungskörper und Eizelle verteilt und letzterer enthält demnach 6 Mischlinge, welche sich je aus einer väterlichen und mütterlichen, oder, da die reife Eizelle bereits eine neue Generation repräsentiert, besser gesagt, aus einer groszväterlichen und groszmütterlichen Hälfte zusammensetzen." To understand fully what this post-maturation gonomery of Häcker involves, it is necessary to review in more detail the processes by which it is attained. In a late prophase of the first maturation mitosis, the twenty-four chromosomes (the somatic number) appear in two rows of six pairs each; the paired bivalent chromosomes having arisen through the union end to end of single chromosomes of the same parental side. Thus, the twelve paternal chromosomes, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l*, are arranged *ab*, *cd*, *ef*, *gh*, *ij*, *kl*, while correspondingly those of the maternal side as *no*, *pq*, *rs*, *tu*, *vw*, *xy*. Each of the pairs then splits longitudinally forming twelve tetrads, six completely paternal and six completely maternal. The first maturation which is equational reduces the tetrads to dyads leaving in the egg twelve bivalent chromosomes, six from each parental side. Now ensues a second synapsis between pairs, this time of

opposite parental sides, thus giving rise to six tetrads, viz;: $\frac{a|b}{n|o}$, $\frac{c|d}{p|q}$, $\frac{e|f}{r|s}$, $\frac{g|h}{t|u}$, $\frac{i|j}{v|w}$, $\frac{k|l}{x|y}$, and through a subsequent rotation these become $\frac{a|n}{b|o}$, $\frac{c|p}{d|q}$, $\frac{e|r}{f|s}$, $\frac{g|t}{h|u}$, $\frac{i|v}{j|w}$, $\frac{k|x}{l|y}$. The second or reducing division distributes these in such a manner that the egg and the second polar body each receives twelve chromosomes (six bivalents) of which six single elements are paternal and six maternal; thus, *an, cp, er, gt, iv, kx,* go to the now matured egg while the rest go to the second polar body.

There is, however, but little probability for such an interpretation as it entirely ignores the significance of the primary synapsis which from the figures of Rückert, '04, and Häcker, '95, himself, undoubtedly takes place at the beginning of oöcytic growth. This undoubtedly results in the reduction of the chromosomes during this period. From the highly probably results of Montgomery and Sutten (which have received strong corroboration from all sides) the twelve bivalents of the first maturation prophase would represent the union of homologous paternal and maternal chromosomes and not, as Häcker assumes, of the same parental side, *i. e.*, paternal with paternal, maternal with maternal On the first of these hypotheses the two ensuing maturation divisions would produce conditions quite analogous to that observed by the great majority of workers on the germ-cells of both animals and plants. But what is far more convincing is the fact, that on the interpretation of Häcker, there would result, in the great majority of cases, an egg with such a chromatic constitution that the potentialities of one group of characters would often be doubled, while those of another group would be entirely lacking. This would not only make Mendelian results impossible but, as Boveri has shown in his work on "Multipolar-mitoses" might lead to the production of defective larvæ.

We must therefore conclude that the segregation of the chromatin in the spermatid of *Pedicellina* is without particular significance and that the gonomery or autonomy of the parental chromatic contributions continues at most only up to the beginning of the growth period of the germs-cells, the mingling having

occurred, in most cases, long before this. At this point, in the processes of synapsis the several chromosomes of the paternal side very probably individually unite with their correspondents on the maternal side. The position of the thus-formed bivalents "in the equatorial plate of the reducing division is purely a matter of chance, that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the position of other pairs and hence, that a large number of combinations of paternal and maternal chromosomes are possible in the matured germ products of an individual" (W. S. Sutton, "The Chromosomes in Heredity," BIOL. BULL., IV., 5).

II.

The nuclei of the several generations of oögonia have already been described (Fig. 3) and need not again be considered except to note that the two nucleoli or the one after the fusion are always homogenous in structure and stain exactly like plastin

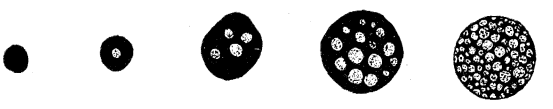

FIG. 12.

bodies. These invariably disappear in the ensuing mitosis. In the youngest oöcytes, a new nucleolus, more rarely two, makes its appearance (Fig. 12, *a*). This body is then quite small and may be situated anywhere within the nucleus; sometimes near the periphery, sometimes on an arm of a chromosomal V or even in the midst of a number of such intersecting arms. It stains intensely at this stage, and shows as yet no trace of vacuolization. In iron-hæmatoxylin, carm-alum, Borel's fluid, etc., it stains more deeply than the chromatin itself and might, if superficially considered, be looked upon as a chromatic nucleolus, as several workers have, on such evidence as this, actually considered it. In Auerbach's fluid, however, the true nature of the body is very clearly brought out. It stains invariably a deep red with the acid fuchsin and shows no trace of the methyl green with which the chromosomes are colored.

As the oöcyte grows, the nucleolus increases in size, and at the same time usually becomes associated with one or more of the chromosomes, and about this period a small vacuole makes its appearance in the center of the body. It stains very slightly and stands out in marked contrast to the darker outer portion (Fig. 12, *b*). From this point onward the course in nucleolar changes is fairly direct and consists in the constant increase of the area of vacuolization until at the end of the period of growth only a minimal portion of the now very large nucleolus shows any stain.

This process of vacuolization may, however, take one of two courses. In the first case, there arise one or more small vacuoles in the immediate vicinity of the first (Fig. 12, *c*), and, with the growth of the nucleolus, the number of these also increases (Fig. 12, *d*), until finally a structure of honey-comb appearance is produced (Fig. 12, *e*). The vacuoles, which fill up the entire space of the nucleolus, are now fairly uniform in size. In the second case the additional vacuoles fuse with the primary one, or the latter may increase in size directly. In either instance, the end result is the same, viz., the central area is much increased in di-

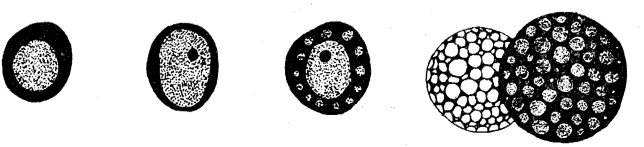

FIG. 13.

ameter, and by the time the yolk makes its appearance in the egg-cytoplasm only a thin outer rim remains of the deeply-staining portion (Fig. 13, *a*, *b*). Vacuoles may, however, later make their appearance in this outer rim (Fig. 13, *c*), showing very clearly that there is no fundamental distinction between the two types above described.

In both these groups, moreover, additional nucleoli often arise secondarily, and these pass through the same processes described for the primary body. In some instances these may fuse, giving rise to a compound nucleolus, one part of which is generally, as is shown by the differences in staining reaction, a little further

advanced than the other (Fig. 13, *d*). In these types also there are often observed certain individual variations which are of interest in their bearing on the relations between the nucleolus and the chromosomes. A cap of deeply-staining material, showing no sign of vacuoles within it, often covers one part of the structure. This varies in size, from the merest rim to a mass half as large as the fully grown nucleolus itself. In some cases there are two such caps to the same nucleolus (Fig. 14, *a*). The other variation consists in the frequent indentation of the outer rim, which gives the appearance of disintegration on the part of the nucleolus (Fig. 14, *b*). This view is made probable by the presence in such cases of large granules, which lie within the indentation and seem directly to fit into it. These conditions, moreover, are found only in advanced stages of egg-development. Altogether, the appearance very strikingly resembles Fig. 13 of

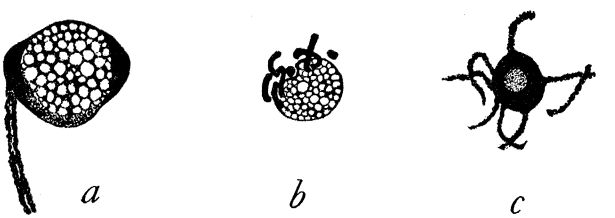

FIG. 14.

Guenther's work, '03, on the nucleolus in the maturing eggs of the Echinodermata (*Holothuria tubulosa*).

This author considers this one of the strongest elements in the evidence, for the chromatic nature of the nucleolus, since the indentation is taken to signify that the nucleolar substance has broken off to become a chromosome. Such a picture as that shown in Fig. 14, *b*, where, in an egg nearly ready for the first maturation, some of the much concentrated chromosomes actually lie within a nucleolar indentation, would most probably be interpreted by Gunther, '03, and some other authors, as very strong evidence in favor of their hypothesis. Indeed, when stained with hæmatoxylin, some of the chromosomes are in such intimate connection with the nucleolus, that all the necessary conditions which their hypothesis demands seem to be realized. Yet nothing can be further from the truth. I have fortunately found such a stage

among my Auerbach preparations, and the results dispel any possibility of confusion. The nucleolus, at this point completely vacuolated, stains intensely red and, in most striking contrast, all the chromosomes lying on its side are as intensely green. It is hardly possible that this striking distinction in staining capacity would exist if there were any more intimate connection between the chromatin and the nucleous, than mere apposition. But more conclusive evidence is given by the fact that the characteristic concentration and localization of the chromosomes, immediately before the first maturation division, is, in most instances, passed through without any relation to the nucleolus. In addition, the history of the chromatin in the growing oöcyte is of a nature entirely incompatable with the conception of the authors cited above. As is pointed out in the section on oögenesis (Dublin, '05), the chromosomes persist, in the reduced number, from the period of synapsis up to the time when they enter into the first maturation spindle. At no stage is there a break in this continuous history. At no stage is a chromatin reticulum formed which might, in some way, make the continued observations of the chromosomes an uncertain task. On this point, the evidence appears to be decisive. These indented nucleoli often occur, when the chromosomes are at the highest point of their extension, as threads, and when even the smallest of them would be many times as large as the space out of which they are supposed to arise. We can now understand the true significance of a condition such as is shown in Fig. 14, *c*, where the chromosomes radiate from the nucleolus and seem to originate from it. This is simply a case, rare enough, where the chromosomes in their extension through the nucleus have crossed such a body. It is probable, further, that they have been intimately associated with the nucleolus by the coagulation incident to the fixing process.

In connection with the deep staining cap-formation on the much vacuolated nucleolus, it is very probable that we are concerned with a phenomena similar in all respects to that observed by Obst, '99, in *Limax*, and in other Molluscs, where a nucleolus was found to arise from the chromatin in the process of concentration. These nucleoli arise late in the oöcytic growth and, in contrast with the cyanophilous nucleoli of the early oöcytes, they

are erythrophilous. In *Pedicellina*, at the stage of the formation of these caps, the chromosomes are in the process of shortening from long ragged threads to small thick rings and bars of the first maturation mitosis, and it is evident that a considerable part of their substance is lost during the change. Fig. 14, *a*, shows very clearly the apposition of one of these long chromosomes to the cap with the strong probability that the substance of the latter is being increased at the expense of that of the former.

As in *Limax* also, the nucleolus of the *Pedicellina* ovum plays no observable part in the formation of the first polar figure. Its structure, now much reduced, disintegrates and its remains are cast out into the light area with which the spindle is surrounded.

The facts observed appear to harmonize, in the main, with the general conclusions which Häcker, '95, has reached. At no period of egg-development is there any possibility for the origin of chromatin from the nucleoli. Indeed, the only perceptible relation which it may have with the chromosomes, excepting those obviously ascribable to accidental apposition, are those where the former adds to its own substance from the cleavage products of the latter. When the uncertain evidence of such works as those of Guenther and Hartman, and the absolute denial given by Häcker, '02, Miss King, '01, and Janssen, '04, to the results of Carnoy and LeBrun, '97–'99, is considered, then it may be concluded that the true nucleoli may, after all, be of one type, and are to be distinguished from the undoubted chromatin bodies such as those described by Blackman, '03, Wilson, '01, and others which are but temporary aggregations of chromatin unwinding the materials from which they are formed.

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DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY,
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Blackman, M. W.

'03 The Spermatogenesis of the Myriopods, II. On the Chromatin in the Spermatocytes of Scolopendra heros. Biol. Bull., V., 4.

Boveri, Th.

'02 Ueber Mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh. d. Phys. Med. ges. zu Würzburg, XXXV.

Carnoy & Lebrun.

'97–'00 La vésicule germinative et les globules polaires chez les Batrachiens. La Cellule, XII, XIV, XVI.

Conklin, E. G.

'01 The Individuality of the Germ Nuclei during the Cleavage of the Egg of Crepidula. Biol. Bull., II., 6.

'02 Karyokinesis and Cytokinesis in the Maturation, Fertilization and Cleavage of Crepidula. Mem. Acad. Nat. Sci. Phila., XII., 11.

Dublin, L. I.

'05 The History of the Germ Cells in Pedicellina americana Annals. N. Y. Acad. Sci., XVI, 1.

Guenther, K.

'03 Ueber den Nucleolus im reifenden Echinodermenei und seine Bedeutung. Zool. Jahrb., XIX., 1.

Häcker, V.

'95 Ueber die Selbständigkeit der väterlichen und mütterlichen Kernbestandtheile während der Embryonalentwicklung von Cyclops. Arch. f. Mik. Anat., XLVI., 4.

'95 Die Vorstadien der Eireifung. Arch. f. Mik. Anat., XLV., 2.

'02 Ueber die Autonomie der väterlichen und mütterlichen Kernsubstanz vom Ei bis zu den Fortpflanzungszellen. Anat. Anz., Bd. XX.

'02 Ueber das Schicksal der elterlichen und grosselterlichen Kernantlile. Jen. Zeitsch., XXXVII.

Hartman, M.

'02 Studien am thierischen Ei. I. Zool. Jahrb., XV.

Herla, V.

'03 Etude des variations de la mitose chez l'ascaride mégalocephale. Arch. de Biol., XIII.

King, H. D.

'01 The Maturation and Fertilization of the Egg of Bufo lentiginosis. Journ. Morph., 17.

Moenkhaus, W. J.

'04 The Development of the Hybrids between Fundulus heteroclitus and Menidia notata, with Especial Reference to the Behavior of the Maternal and Paternal Chromatin. Journ. Anat., 3, 1.

Montgomery, T. H.

'04 Some Observations and Considerations Upon the Maturation Phenomena of the Germ Cells. Biol. Bull., VI., 3.

Obst, P.

'99 Untersuchungen über das Verhalten der Nucleolen, etc. Zeitsch. f. Wiss. Zool., LXVI.

Rückert, J.

'94 Zur Eireifung bei Copepoden. Anat., Hefte 4.

'95 Ueber das Sebständigbleiben der väterlichen und mütterlichen Kernsubstanz während der ersten Entwicklung des befruchteten Cyclops-Eies. Arch. f. Mik. Anat., XLV.

Sutton, W. S.

'03 The Chromosomes in Heredity. Biol. Bull., V., 4.

Wilson, E. B.

'01 Experimental Studies in Cytology, I. A Cytological Study of Artificial Parthenogenesis in Sea-Urchin Eggs. Arch. f. Entwm., XII.

Zoja, R.

'95 Sulla independenza della cromatina paterna e materna nel nucleo, etc. Anat. Anz., XI.